Remarks

In response to the Notice to Comply dated July 3, 2001, we respectfully submit that the application as amended herein complies with the requirements of the sequence rules set forth in 37 CFR 1.821 - 1.825 for the reasons detailed hereinafter.

The Specification has been amended according to the kind suggestion of the Examiner to include in the brief descriptions of the figures the sequence identification numbers assigned to the sequences in the sequence listing. Specifically, the brief description of Figure 12 on page 8 of the Specification has been amended to include SEQ. ID. Nos. 51, 52, 53, and 54 corresponding to the sequences of the 16S rRNA, *cspA* mRNA, *cspB* mRNA, and *cspG* mRNA, respectively.

Similarly, the brief description of Figure 14 on pages 9-10 of the Specification has been amended to identify the SEQ. ID. NOs. corresponding to the *cspA* 5'-UTR deletion constructs pMM67, pMM022, pMM023, pMM024, pMM025, and pMM026 as SEQ. ID. NOs. 55-60, respectively.

Additionally, the brief description of Figure 15A on page 10 of the Specification as amended specifies the *cspB*-DB sequence as SEQ. ID. NO. 61 and nucleotides 1481-1443 of 16S rRNA as SEQ. ID. NO. 62.

The brief description of Figure 16 as amended on page 11 of the Specification identifies the SEQ. ID. NOs. of the DB sequences of pJJG78DB1 and pJJG78DB2 as SEQ. ID. NOs. 64 and 65, respectively, in addition to the 16S rRNA anti-DB sequence as SEQ. ID. NO. 63.

The brief description of Figure 17B on pages 11-12 of the Specification has been amended to identify the SEQ. ID. NOs. of pJJG78, 16S rRNA anti-DB, pINZDB1, pINZDB2, pINZDB3, and pINZDB4 as SEQ. ID. NOs. 66-71, respectively.

The claims have been similarly amended. Specificially, Claim 5 as amended

identifies the sequence corresponding to nucleotides 1 to 11 of the *cspA* 5'-UTR as nucleotides 1 to 11 of SEQ. ID. NO. 55. Claim 10 as amended specifies the sequence corresponding to nucleotides 56 to 117 of the *cspA* 5'-UTR as nucleotides 56 to 117 of SEQ. ID. NO. 55. Likewise, Claim 14 as amended identifies the sequence corresponding to nucleotides 123 to 135 of the *cspA* 5'-UTR as nucleotides 123 to 135 of SEQ. ID. NO. 55.

We respectfully submit that Claims 3 and 13 do not require SEQ. ID. NOs., as those claims recite a gene or element of a gene as distinguished from a specific nucleotide sequence within a gene.

In light of the foregoing, we respectfully request early action on the merits.

Respectfully submitted,

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Versions with Markings to Show Changes Made to the Specification

Please replace the paragraph bridging page 8 to page 9 with the following:

Fig. 12 shows sequence similarities of *cspA* (SEQ. ID. NO. 52), *cspB* (SEQ. ID. NO. 53), *cspG* (SEQ. ID. NO. 54) and *cspI* mRNAs around the SD sequence and potential base pairing between the *cspA* mRNA and 16S rRNA (SEQ. ID. NO. 51). Nucleotide numbers of *cspA* (Tanabe *et al.*, 1992), *cspB* (Etchegaray *et al.*, 1996), *cspG* (Nakashima *et al.*, 1996), and *cspI* mRNA (Wang *et al.*, 1999) are given starting from the major transcription initiation site as +1. The sequence of 16S rRNA is from Brosius *et al.*, 1978. Nucleotides identical in the three *csp* mRNAs are shown in bold letters. The 13-base homologous sequence is *cspA, cspB, cspG,* and *cspI* are boxed (the upstream box). Positions of the SD sequence and the initiation codon are underlined. Potential base pairing between *cspA* mRNA and 16S rRNA are indicated by vertical lines. Positions of RNase V1 sensitive sites (Powers *et al.*, 1988) are dotted.

Please replace the last paragraph on Page 9 with the following:

Fig. 14 shows a comparison of the secondary structures of the 5'-UTRs for the deletion constructs. Secondary structures of the 5'UTR for each deletion construct ~~was~~ were predicted with a nucleotide sequence analysis program (DNASIS-Mac; Hitachi Software Engineering Co. Ltd.) based on the method of Zuker and Stieger, 1982. Nucleotides are numbered as the position in the *cspA* mRNA starting from the transcription initiation site as +1. The position of the deletion in each mutant is shown by an arrow with the nucleotide numbers of the deleted region. The highly conserved 13-base sequence upstream of the SD sequence designated the upstream box are boxed. The initiation codon and the SD sequence are also boxed. The *cspA* 5'UTR deletion constructs: (A) pMM67 (SEQ. ID. NO. 55); (B) pMM022 (SEQ. ID.NO. 56); (C) pMM023 (SEQ. ID. NO. 57); (D) pMM024 (SEQ. ID.

NO. 58); (E) pMM025 (SEQ. ID. NO. 59); and (F) pMM026 (SEQ. ID. NO. 60).

Please replace the paragraph corresponding to the first paragraph on page 10 with the following:

Fig. 15 shows enhancement of *cspB* translation by DB. (A) *cspB*-DB-anti-DB complementarity: the *cspB*-DB sequence (SEQ. ID. NO. 61) is boxed and emcompasses the region from codons 5 to 9 (Mitta et al., 1997). Nucleotides 1481-1443 of 16S rRNA (SEQ. ID. NO. 62) are shown. Additional *cspB* mRNA-16S rRNA possible base pairings downstream of DB are also shown. The AUG codon is circled, the SD sequence is boxed and L-shaped arrows show the positions where the *cspB* gene was fused to lacZ. **(B)** Translational *cspB-lacZ* fusion constructs. On the top, the *E. coli cspB* gene is depicted from its 5' end. In pB3, pB13 and pB17, the *lacZ* gene is fused to *cspB* at residue +177 (3 aa), +200 (13 aa) and +212 (17aa), respectively. The pB13sd and pB17sd are the same as pB13 and pB17, respectively, except that their SD sequences are changed from 5'-AGGA-3' to 5'-CTTC-3'. **(C)** β-galactosidase activity of the *cspB-lacZ* constructs obtained before (time 0) and after (1, 2 and 3 hr) temperature shift from 37 to 15°C. *E. coli* AR137 cells were transformed with pB3, pB13, pB13sd, pB17 and pB17sd were grown in medium, and at mid-log phase (OD_{600} = 0.4) cultures were shifted from 37 to 15°C. β-galactosidase activity was measured. **(D)** mRNA levels of pB3, pB13, pB17 or pB13sd after temperature shift from 37 to 15°C: the *cspB-lacZ* mRNAs were detected by primer extension before temperature downshift (time 0) and at 1, 2 and 3 hrs after temperature shift. **(E)** mRNA stability from pB3, pB13, pB17 and pB13sd:*E. coli* AR137 cells transformed with pB3, pB13, pB17 and pB13sd were grown under the same conditions described above. At mid log phase, the culture was shifted to 15°C and after 30 min., rifampicin was added to a final concentration of 0.2 mg/ml (time 0). Total RNA was extracted at 5, 10 and 20 min. after

rifampicin addition. The *cspB-lacZ* mRNAs were detected by primer extension.

Please replace the paragraph bridging pages 10 to 11 with the following:

Fig. 16 shows the effect of a perfectly matching DB enhancing the translation of *cspA*. **(A)** Translational *cspA-lacZ* fusion constructs. The *cspA* gene structure from its 5'-end is ~~showed~~shown at the top. pJJG78DB1 and pJJG78DB2 were constructed from pJJG78 as described in Experimental Procedures. The DB sequences of pJJG78DB1 (SEQ. ID. NO. 64) (12 matches) and pJJG78DB2 (SEQ. ID. NO. 65) (15 matches) are shown at the bottom with the 16S rRNA anti-DB sequence (SEQ. ID. NO. 63). **(B)** β-Galactosidase activity of the *cspA-lacZ* fusion constructs after cold shock at 15°C. *E. coli* AR137 cells transformed with pJJG78, pJJG78DB1 or pJJG78DB2 were grown in LB medium, and at mid-log phase (OD_{600}=0.4) cultures were shifted from 37°C to 15°C. β-galactosidase activity was measured before (time 0) and 1, 2 and 3 hr after the shift. **(C)** Detection of the *cspA-lazZ* mRNAs. Total RNA from *E. coli* AR137 cells carrying pJJG78, pJJG78DB1 or pJJG78DB2 was extracted at the same time points indicated above and used as a template for primer extension. **(D)** mRNA stability from the *cspA-lacZ* constructs. *E. coli* AR137 cells transformed with pJJG78, pJJG78DB1 and pJJG78DB2 were grown as described above. At mid-log phase, the cultures were shifted to 15°C and after 30 minutes rifampicin was added to a final concentration of 0.2 mg/ml (time 0). Total RNA was extracted at 5, 10 and 40 minutes after rifampicin addition. The *cspA-lacZ* mRNAs were detected by primer extension.

Please replace the paragraph bridging pages 11 to 12 with the following:

Fig. 17 shows that a perfectly matching DB enhances translation at 37°C: **(A)** pIN-*lacZ* constructs. The *XbaI-SalI* fragment from pJJG78 or pJJG78DB2 was inserted into the *XbaI-*

SalI sites of pIN-III to create pINZ and pINZDB1, respectively which then were used to create pINZDB2, pINZDB3 and pINZDB4.

(B) mRNA sequences of the pIN-*lacZ* constructs showing the position of SD, AUG and DB. The *lacZ* in pJJG78 has a 10-match DB. The perfect match DB located after the 5th codon has 16 residues complementary with the anti-DB. The pin-*lacZ* constructs: pINZDB1 (SEQ. ID. NO. 68); pINZDB2 (SEQ. ID. NO. 69); pINZDB3 (SEQ. ID. NO. 70); and pINZDB4 (SEQ. ID. NO. 71). 16S rRNA anti-DB (SEQ. ID. NO. 67). pJJG78 (SEQ. ID. NO. 66).

(BC) β-Galactosidase activity of the pINZ-*lacZ* constructs. Cultures of *E. coli* AR137 cells transformed with pINZ, pINZDB1, pINZDB2, pINZDB3 and pINZDB4 were grown at 37°C under the same conditions described in Figure 1. IPTG (1 mM) was added at mid-log phase to each culture. β-Galactosidase activity was measured before (time 0) and at 0.5, 1, 2 and 3 hr after IPTG addition.

~~**(C)** mRNA sequences of the pIN-*lacZ* constructs showing the position of SD, AUG and DB. The *lacZ* in pJJG78 has a 10-match DB. The perfect match DB located after the 5th codon has 16 residues complementary with the anti-DB.~~ **(D)** ~~shows the rate~~Rate of β-galactosidase synthesis of the pINZ-*lacZ* constructs. Cultures of *E. coli* AR137 cells carrying pINZ or pINZDB1 were grown at 37°C under the same conditions described above. IPTG (1mM) was added at mid-log phase to each culture. Rate of β-galactosidase synthesis was measured before (time 0) and 0.5, 1, 2, 3 and 4 hr after IPTG addition. Cells were pulse-labeled with trans-[^{35}S]-methionine. Cell extracts from each time point were analyzed by 5% SDS-PAGE and the β-galactosidase synthesis was measured by phosphorimager. The ratio of β-galactosidase synthesis of pINZ and pINZDB1 is shown at each time point.

Version with Markings to Show Changes Made to the Claims

5. (Amended) The nucleic acid molecule of Claim 3, wherein said 5'-UTR comprises nucleotides +1 to +11 of the *cspA* 5'-UTR (nucleotides 1 to 11 of SEQ. ID. NO. 55) or a ~~subststantially homologous sequense thereof.~~nucleotide sequence having substantial homology to nucleotides +1 to +11 of the *cspA* 5'-UTR (nucleotides 1 to 11 of SEQ. ID. NO. 55).

10. (Amended) The nucleic acid molecule of Claim 8 wherein said cold-shock inducible gene comprises nucleotides +56 to +117 of the *cspA* 5'-UTR (nucleotides 56 to 117 of SEQ. ID. NO. 55) or a ~~nucleotides~~nucleotide sequence having substantial homology to nucleotides +56 to +117 of the *cspA* 5'-UTR (nucleotides 56 to 117 of SEQ. ID. NO. 55).

14. (Amended) The nucleic acid molecule of Claim 13, comprising nucleotides +123 to +135 of the *cspA* 5'-UTR (nucleotides 123 to 135 of SEQ. ID. NO. 55) or ~~nucleotides~~a nucleotide sequence having substantial homology to nucleotides +123 to +135 of the *cspA* 5'-UTR (nucleotides 123 to 135 of SEQ. ID. NO. 55).